PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Judith E Meis
Serial No.:	09/979,518
Filing Date:	04/10/2002
Title:	*Reverse Transcription Activity from Bacillus Stearothermophilus DNA Polymerase in the Presence of Magnesium*
Art Unit:	1652
Conf. No.	6310
Examiner:	Richard G. Hutson
Atty. Docket:	310307.90134

DECLARATION UNDER 37 CFR §1.131/1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, Gary Dahl, on oath say and declare that:

1. I, Gary Dahl, am a co-founder, President and CEO of EPICENTRE Technologies Corporation, the assignee of the above-referenced application. As such, I am intimately familiar with the above-identified patent application and the subject matter claimed therein, and I make this declaration in support of that patent application.

2. EPICENTRE Technologies Corporation, assignee of the above-identified application, was founded in 1987 to create, develop and market new molecular biology enzymes that provide improved performance for biomedical research applications through a process of continued innovation, application and commercialization.

3. I have a Ph.D. degree with joint majors in Oncology and Botany from the University of Wisconsin, Madison, Wisconsin, and I have more than 30 years of experience working in the field of molecular biology, including more than 25 years of industrial experience working in the field of molecular biology reagents, primarily involving molecular biology enzymes. I have participated in and supervised work on numerous projects to find and develop commercially useful molecular biology enzymes.

4. I have reviewed the September 28, 2009 Final Office Action pending in this Application. I understand that the Examiner has rejected claims 60, 61, 63, 64, 66 and 67 as being unpatentable over Roche Molecular Biochemicals Catalog, 1999, pages 50-51; Sellman et al. Journal of Bacteriology, Vol 174, No. 13, pages 4350-4355; and Lu et al. BioFeedback, Vol 11, No. 4, pages 464-466, 1991. The Examiner stated that "the substitution of the Bst DNA polymerases taught by Sellman et al. or Lu et al. into the methods taught by Roche Molecular Biochemicals Catalog, 1999 would yield predictable results." (2009/9/28 Final Office Action, pg. 8). This Declaration is offered to provide evidence addressing the Examiner's conclusion above.

5. In order to explore the veracity of the Examiner's conclusion that the results of substitution of a DNA polymerase from a different moderately thermophilic bacterium, such as Bst, into the methods taught by Roche Molecular Biochemicals Catalog, 1999 was predictable and would yield the same results identified and claimed by the Applicant of the present application, I supervised the testing of three other commercially available DNA polymerases derived from similar moderately thermophilic bacteria for their respective reverse transcriptase activities. The three DNA polymerases chosen were:

(1) Bst DNA polymerase Large Fragment from New England BioLabs, Catalog Number: M0275M; (2) BcaBEST™ DNA polymerase from Takara Bio Inc.'s BcaBEST™ RNA PCR Kit Ver.1.1, Catalog Number: RR023A; and (3) DisplaceAce™ DNA polymerase from EPICENTRE Biotechnologies, Catalog Number: D08061K, which is a truncated DNA polymerase derived from *Geobacillus kaustophilus*.

6. The reverse transcriptase activities of each of the three enzymes were assayed using methods similar to those described in the present application: (1) in the presence of manganese cations; and (2) in the presence of magnesium cations in the absence of manganese cations. Briefly, each of the tested DNA polymerases was incubated for 30 minutes at temperatures between 40-60 degrees C in a reverse transcription reaction mixture containing an RNA template, a primer complementary to the 3'-end of the template, all 4 deoxyribonucleoside triphosphates, and either only Mn^{2+} cations or only Mg^{2+} cations. Then, an aliquot of each reverse transcription reaction mixture was analyzed for the presence or absence of a first-strand cDNA band by electrophoresis on a denaturing agarose gel and staining the gel with SYBR® Gold dye to visualize the bands.

7. Results of the reverse transcriptase assays were as follows:

Enzyme	Source	cDNA Band Synthesized in Presence of Only:	
		Magnesium	Manganese
Bst DNA Polymerase Large Fragment	New England BioLabs	NO	YES
BcaBest™ DNA Polymerase	Takara Bio Inc.	YES	YES
DisplaceAce™ DNA polymerase	EPICENTRE Biotechnologies	NO	YES

8. These results demonstrate very clearly that "the substitution of the Bst DNA polymerases taught by Sellman et al. or Lu et al. into the methods taught by Roche Molecular Biochemicals Catalog, 1999 did not yield the predictable reverse transcriptase activity in the presence of only Mg^{2+} cations, as alleged by the Examiner. As shown in the table, although all of the commercially available DNA polymerases tested had reverse transcriptase activity in the presence of Mn^{2+} cations, only one of the three DNA polymerases had reverse transcriptase activity in the presence of only Mg^{2+} cations in the absence of Mn^{2+} cations.

9. In particular, although the presently pending application disclosed DNA polymerases from two Bst strains that have reverse transcriptase activity in the presence of only Mg^{2+} cations, the commercially available Bst DNA polymerase large fragment from New England Biolabs did not show activity in synthesizing a cDNA band that could be visualized on a gel in these assays. Thus, it is very clear from these experiments that it is not obvious that one of skill could substitute the Bst polymerases of Sellman or Lu into the methods of Roche with any predictable chance of success. Further, these results show that it is not obvious which Bst polymerases will have reverse trancriptase activity in the presence of Mg^{2+} cations and in the absence of Mn^{2+} cations. The results also teach that the presently claimed methods are specific to the cited Bst DNA polymerases from particular strains of bacteria and that this activity cannot even be predicted to be present in DNA polymerases from all bacterial strains comprising a single genus and species category (e.g., all Bst strains), at least based on current categories of bacterial nomenclature.

10. I hereby declare all statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and the such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated this 27th day of February, 2010.

Gary Dahl

Cdxzt5rf